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Evolutionary algorithms to solve complicated NMR spectra

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The complexity of ^1H NMR spectra of solutes in partially ordered solvents such as liquid crystals increases rapidly with the number of spins. Spectra of simple solutes with sufficient symmetry and containing not too many spins (typically ≤ 8) are readily analyzed. The analysis of larger spin systems is more difficult, and often impossible. In this paper we present the application of a general automated evolutionary algorithm to solve the highly complex proton NMR spectrum of the 12-spin system pentane, a solute that interconverts rapidly among several symmetry-unrelated conformations. The interpretation of the spectral parameters that are obtained from the analysis requires the use of a model to connect relative orientational orders in symmetry-unrelated conformers. © 2009 American Institute of Physics. [DOI: 10.1063/1.3061622]

I. INTRODUCTION

The NMR spectroscopy of orientationally ordered solutes in anisotropic liquids yields a wealth of information about both the solute and the intermolecular interactions between the solute and the solvent.^{1,2} The extension to solutes that can exist in several symmetry-unrelated conformers is both interesting and challenging. The conformer distribution in such flexible molecules is governed by the total potential which in the condensed phase includes both intra- and intermolecular terms. This distribution can also be affected by orientational ordering. The extent of both isotropic and anisotropic effects in the solvent is difficult to measure. Because dipolar couplings between nuclei on different rigid subunits of a flexible molecule are very sensitive to conformer geometry and orientational order, they offer an excellent route to gaining insight into this difficult problem. Up to now the measurement of such dipolar couplings has been hampered by the difficulty of analysis of the complicated spectra that are obtained. Two severe problems inhibit such studies.

First, the NMR spectra that we consider are weighted averages over all available conformers. The anisotropic spectral parameters involve a sum over terms, each of which is the product of a conformer probability times a conformer order parameter, and it is not possible to separate terms in the product. Hence some assumption must be made about either conformer probabilities or order parameters. In order to extract information about probabilities, below we consider ways of modeling how order parameters change with conformer change.

The second problem is the complexity of spectra obtained from orientationally ordered solutes that contain more than about eight spins. Various approaches have been applied to the solution of such spectra. Multiple-quantum NMR spectroscopy^{3,4} has been used for the analysis of quite complicated spin systems, but the nature of the technique leads to spectral parameters that have large associated errors; however, accurate spectral parameters can be obtained using the inaccurate Multiple-quantum parameters as initial guesses for the analysis of the more precise single-quantum high-resolution spectrum.^{5,6} In an alternative approach, two methods that deploy deuterium isotopic substitution have been used. In the first, order parameters are determined for each C–D bond from the deuterium NMR spectrum of the orientationally ordered species.^{7,8} These order parameters are then used to test models for the orientational order weighted over all conformers. One drawback is that the deuterium couplings belong to a rigid molecular segment and their values give order parameters of the C–D bond averaged over all conformers—the intrinsic coupling is not directly dependent on conformer. On the other hand, proton-proton couplings between different rigid molecular segments do depend on conformer, and in this sense are much more informative quantities for conformational analysis. In a second two-dimensional deuterium NMR method, deuterium decoupling is used to obtain proton-proton dipolar couplings between pairs of protons remaining on the largely deuterated species.⁹ Unfortunately, in some cases only a limited number of dipolar couplings has been determined, and the accuracy is again less than that expected from the analysis of a high-resolution spectrum.

The idea of the automatic analysis of complicated NMR spectra of solutes in liquid crystals has been explored over the years, and much progress has been made.^{10–17} However,

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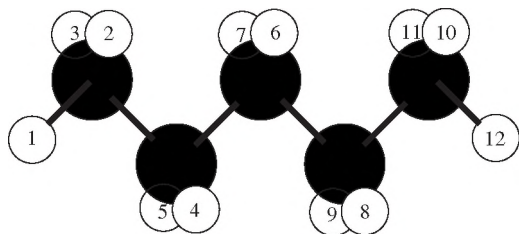


FIG. 1. The all *trans* conformer of pentane showing the labeling of the protons.

the complexity of spectra analyzed so far has not yet surpassed that of spectra that could be solved by conventional means. Often the algorithms used require a substantial degree of operator intervention in order to avoid false minima and have been limited to moderately complicated NMR spectra that contain a thousand or so lines. More recently evolutionary algorithms (EAs) have been applied to such automatic analysis of NMR spectra.^{18–20} The first attempts required significant operator input and encountered problems with false minima.^{18,19} A more sophisticated approach that avoids the problem of false minima has already shown great

promise of yielding accurate spectral fits with a minimum of operator intervention.²⁰ This recent success in which the genetic algorithm (GA) has been applied has prompted us to explore the analysis of the very complicated spectra of pentane as a solute in nematic liquid-crystal solvents.

Pentane (Fig. 1) is a molecule with interesting conformational features and an ideal model compound for conformational studies by NMR. The proton NMR spectrum of orientationally ordered pentane is very complicated (consisting of roughly 20 000 transitions with many overlapping lines and few distinguishing features, Fig. 2) making it essentially impossible to analyze by conventional means.

The purpose of the present paper is twofold. First we perform a sophisticated EA analysis on ^1H NMR spectra of pentane dissolved in two different nematic phases to obtain very accurate sets of dipolar couplings. Second, we use these experimental dipolar couplings for a preliminary conformational analysis of the solute. In order to estimate the change in orientational order with change in conformer, we employ phenomenological models for the orientational potential that are based on solute size and shape.

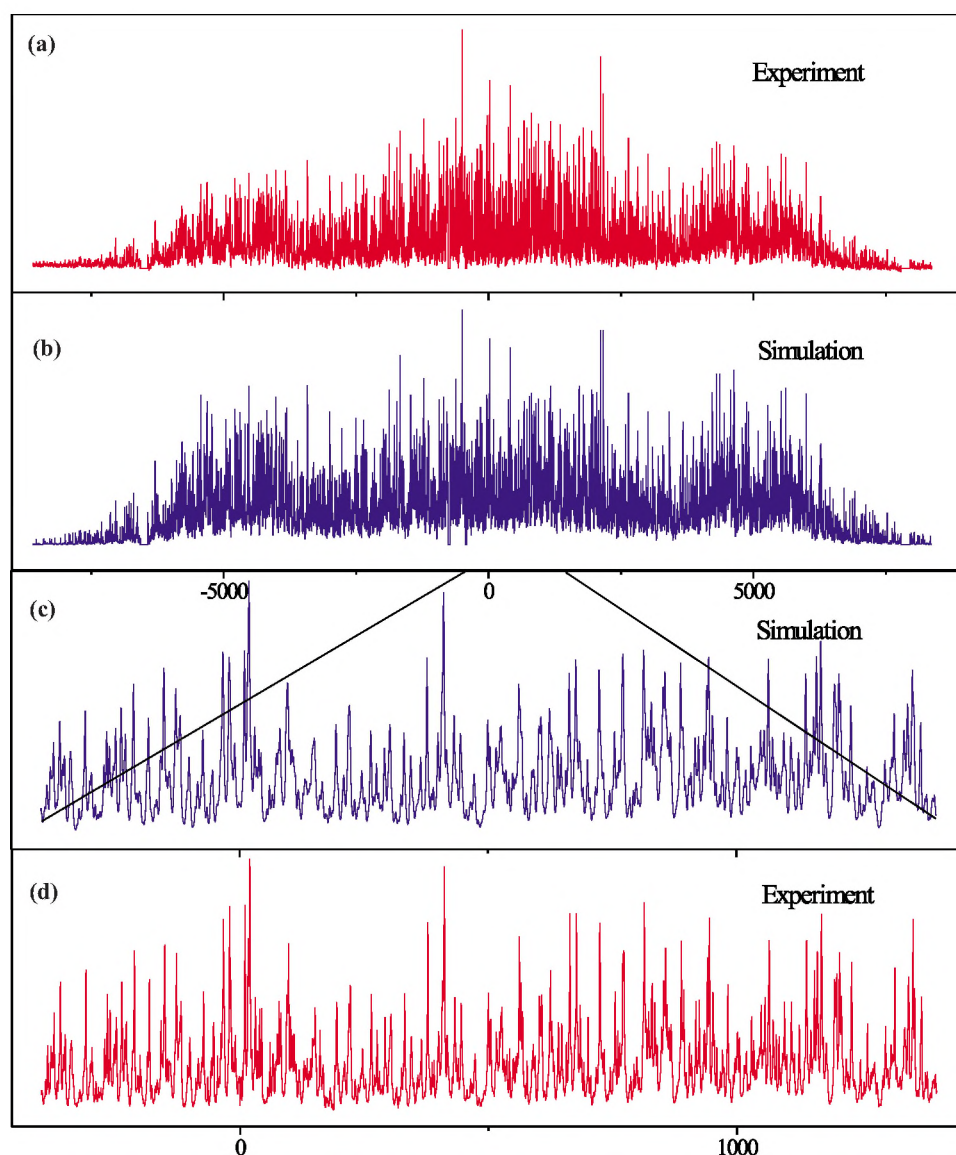


FIG. 2. (Color online) ^1H NMR spectrum of pentane dissolved in 1132. The experimental spectrum (a) is compared with the final fit obtained by varying 11 dipolar couplings, 9 indirect spin-spin couplings, and 3 chemical shieldings (b); in the next frames the spectrum is enlarged to show the experimental spectrum (d) and the same fit as above (c). All horizontal scales are in Hz. The 11 indirect spin-spin couplings were varied only in the final fitting procedure; in earlier iterations they were given their isotropic values.

II. EXPERIMENTAL

Approximately 5 mol % of *n*-pentane was dissolved in each of the liquid crystals Merck ZLI-1132 (1132) and a “magic mixture” of 55 wt% 1132 and 45 wt% *p*-ethoxybenzylidene-*p'*-*n*-butylaniline and the liquid-crystal solutions were placed into 5 mm outer diameter NMR tubes. About 0.5 mol % 1,3,5-trichlorobenzene was added to serve as an orientational reference. After thoroughly mixing the sample in the isotropic phase, a capillary tube filled with acetone- d_6 was placed into the NMR tube and centered with the help of teflon spacers. The acetone- d_6 provides a lock signal for Z-axis shimming and time averaging. After sealing the tube, experiments were carried out with a Bruker-400 high-resolution NMR spectrometer equipped with an inverse detection probe. After allowing sufficient time for temperature equilibration, single-quantum high-resolution proton NMR spectra were accumulated over a 10 min period at 298.46 K while spinning the sample tube at 30 Hz. The spectral digital resolution and number of points collected were 0.64 Hz, 32 768 points (1132) and 0.29 Hz, 45 328 points (magic mixture).

Since the EAs iterate on intensity as well as frequency, it was necessary to remove the broad liquid-crystal background upon which the *n*-pentane transitions are superimposed. To do this, samples were made up that were identical in all respects to the first ones, but without the *n*-pentane. Since the nematic-isotropic phase transition temperatures and hence the liquid-crystal order parameters are affected by solute concentration, it was necessary to adjust temperature in order to collect a spectrum of the “blank” that matched the liquid-crystal “baseline,” i.e., with the same spectral features and width as the sample of interest. This temperature turned out to be 303.8 K for the 1132 sample. Care was taken to match the scaling and phasing of the two spectra. Using the Bruker NMR software TOP SPIN, the blank spectrum was scaled and subtracted from the spectrum of interest in order to remove the liquid-crystal “background” signal and obtain the spectrum of partially oriented *n*-pentane.

III. THEORETICAL CONSIDERATIONS

The Hamiltonian (in Hz) of a molecule dissolved in a uniaxial nematic liquid crystal is given by

$$H = \sum_{\mu} \nu_{\mu} I_{Z,\mu} + \sum_{\mu < \nu} J_{\mu\nu} \mathbf{I}_{\mu} \cdot \mathbf{I}_{\nu} + \sum_{\mu < \nu} D_{\mu\nu} (3I_{Z,\mu} I_{Z,\nu} - \mathbf{I}_{\mu} \cdot \mathbf{I}_{\nu}), \quad (1)$$

where ν_{μ} is the resonance frequency of nucleus μ and $J_{\mu\nu}$ and $D_{\mu\nu}$ are the indirect spin-spin and the direct dipole-dipole coupling between nuclei μ and ν . The chemical shielding term ν_{μ} can have both isotropic and anisotropic parts, whereas the $D_{\mu\nu}$ terms are completely anisotropic and hence do not contribute to the familiar spectra of isotropic liquids. We omit effects of the anisotropy in $J_{\mu\nu}$ which are to modify slightly the observed values of $D_{\mu\nu}$ and which are negligible for proton-proton couplings.

The dipolar coupling between nuclei μ and ν is

$$D_{\mu\nu} = -\frac{h\gamma_{\mu}\gamma_{\nu}}{4\pi^2} \left\langle r_{\mu\nu}^{-3} \left(\frac{3}{2} \cos^2 \theta_{\mu\nu,Z} - \frac{1}{2} \right) \right\rangle, \quad (2)$$

where $r_{\mu\nu}$ is the internuclear separation and $\theta_{\mu\nu,Z}$ is the angle between the $\mu\nu$ and magnetic field directions, and the angle brackets denote an average over solute internal and reorientational motions. With the assumption that the molecule rapidly interconverts among several “rigid” conformers, Eq. (2) can be written as

$$D_{\mu\nu} = -\frac{h\gamma_{\mu}\gamma_{\nu}}{4\pi^2} \sum_i p_i r_{i,\mu\nu}^{-3} \sum_{k,l} S_{i,kl} \cos \theta_{\mu\nu,k} \cos \theta_{\mu\nu,l}, \quad (3)$$

where i labels the conformer and $\theta_{\mu\nu,k}$ is the angle between the conformer-fixed k axis and the vector joining nuclei μ and ν which have internuclear separation $r_{\mu\nu}$. $S_{i,kl}$ is the kl element of the order parameter tensor for conformer i ,

$$S_{i,kl} = \left\langle \frac{3}{2} \cos \theta_{k,Z} \cos \theta_{l,Z} - \frac{1}{2} \delta_{kl} \right\rangle, \quad (4)$$

where the angle brackets denote an average over conformer reorientational motions and $\theta_{k,Z}$ is the angle between the conformer-fixed k axis and the space-fixed Z axis, which for the liquid crystals used here lies along both the director and magnetic field directions. In this picture small-amplitude vibrational corrections and vibration-reorientation coupling are neglected (vibration-reorientation coupling will be included later in a phenomenological fashion).

The conformer probabilities are given by^{5,7}

$$p_i = \frac{G_i \exp\left(-\frac{U_i^{\text{iso}}}{k_B T}\right) \int \exp\left(-\frac{U_i^{\text{aniso}}(\Omega)}{k_B T}\right) d\Omega}{\sum_j G_j \exp\left(-\frac{U_j^{\text{iso}}}{k_B T}\right) \int \exp\left(-\frac{U_j^{\text{aniso}}(\Omega)}{k_B T}\right) d\Omega}, \quad (5)$$

with

$$\sum_i p_i = 1, \quad (6)$$

$G_i = \sqrt{I_{i,xx} I_{i,yy} I_{i,zz}}$ is a rotational kinetic energy factor that depends on the moment of inertia tensor $I_{i,\alpha\alpha}$ for each conformer i and

$$U_i^{\text{iso}} = U_{i,\text{int}}^{\text{iso}} + U_{i,\text{ext}}^{\text{iso}} \quad (7)$$

includes the internal energy $U_{i,\text{int}}^{\text{iso}}$ associated with the conformer and the isotropic part $U_{i,\text{ext}}^{\text{iso}}$ of the interaction energy between the conformer and the external field. $U_i^{\text{aniso}}(\Omega)$ is the orientationally dependent interaction energy between the conformer and the external field. If we have some theory or model for $U_i^{\text{aniso}}(\Omega)$, we can calculate the conformer order parameters from

$$S_{i,kl} = \frac{\int \left(\frac{3}{2} \cos \theta_{k,Z} \cos \theta_{l,Z} - \frac{1}{2} \delta_{kl} \right) \exp\left(-\frac{U_i^{\text{aniso}}(\Omega)}{k_B T}\right) d\Omega}{\int \exp\left(-\frac{U_i^{\text{aniso}}(\Omega)}{k_B T}\right) d\Omega}. \quad (8)$$

IV. SPECTRAL ANALYSIS USING EVOLUTIONARY ALGORITHMS

The ^1H NMR spectrum of pentane in Fig. 2 has no easily recognizable patterns that could aid the spectral analysis. A visual inspection of the spectrum gives no clue whatsoever of the magnitudes of the dipolar couplings. When applying an EA to such a problem it should be realized that the computational time increases very steeply with the search ranges used for every parameter. It is therefore prudent to choose initial values and ranges for each parameter that are as narrow as possible, but still wide enough to include the correct value. In the following we shall describe a strategy to do just that.

High-resolution NMR spectral values are used for indirect spin-spin couplings which are initially assumed to remain unchanged in the spectrum of the orientationally ordered solute. High-resolution NMR values are also used as initial estimates of chemical shifts, and the search ranges were chosen to be consistent with centering the calculated spectrum to the experimental one.

Initial estimates of the 11 independent dipolar couplings are more problematic because they depend on the structures, order matrices, and conformer probabilities. The following approach was used. We assume that the conformer problem can be described adequately by the rotamer isomeric state (RIS) approximation²¹ in which the CCCC dihedral angle of each $\text{CH}_2\text{--CH}_2$ bond can exist in one of the three local arrangements: *trans* (t), *gauche* plus (g_+) or *gauche* minus (g_-). For pentane there are then nine RIS conformers, some being related by symmetry. The four independent conformers and their symmetry are tt (C_{2v} , singly degenerate), tg (C_1 , four-fold degenerate), g_+g_+ (C_2 , two-fold degenerate), and g_+g_- (C_s , two-fold degenerate). It is necessary to assign values for conformer probabilities. The extra energy involved in a *gauche* conformer E_{tg} was set to the value of 650 cal, with the weight of the g_+g_- conformer set to zero because of the additional energy involved with the steric interaction of the two methyl groups in this configuration (the “pentane” effect).²¹ For the structure of each conformer we used the same bond angles and bond lengths that were used for a previous analysis of butane.⁵

Finally, we require estimates of the pentane conformer order matrices in the two liquid-crystal solvents magic mixture and 1132. Solute orientational order in the magic mixture is dominated by a single orientational mechanism that depends on solute size and shape alone.^{1,2} Hence, phenomenological size and shape models are appropriate for prediction of conformer order parameters in this case. For 1132 the situation is somewhat different because at least one additional mechanism that depends on the anisotropic electrostatic properties of the solute contributes to the orientational potential. However, in the case of alkanes these additional mechanisms are considered to be of minor importance and can be neglected.²² Thus the moniker “magic solute” is often used for alkanes. Hence, size and shape models (with the interaction constant adjusted to account for changes in liquid-crystal solvent and temperature) are probably also appropriate to describe the orientational order of pentane in

1132. These considerations are supported by literature data on propane²³ for which the ratios of the two propane order parameters are approximately equal in the liquid crystals magic mixture and 1132.

Short-range size and shape interactions have been modeled quite successfully with the phenomenological circumference/integral (CI) size and shape model,²⁴ leading to order parameters that are predicted to better than 10%.¹ Hence, we use this model to provide estimates of pentane conformer order matrices. We fix the ratio of the two interaction parameters of the model to the value of 23.529 found in an earlier study of 46 solutes in the magic mixture.²⁴ The value chosen for the one remaining parameter k is the one that leads to a calculated pentane spectrum that possesses the same width as the observed one.

Spectra calculated using the order parameters estimated above bore resemblance to the experimental ones in the sense that differences were appreciable, but the overall underlying features were reasonable. An initial, unsuccessful attempt with the GA fitting procedure was performed using (and varying within reasonable limits) the above chemical shifts, order parameters for each conformer, and conformer probabilities. Such parameters cannot be expected to give an exact solution because they are based on models and guesses, and hence may have significant uncertainties. The search was abandoned in favor of one that varied the 11 independent dipolar couplings and three chemical shifts that could, in principle, yield an exact match to the experimental spectrum. Indeed there are fewer independent dipolar couplings than conformer order parameters, giving further justification for basing the second attempt on the dipolar couplings.

In order to estimate reasonable initial values and ranges for the dipolar couplings, spectra were calculated for various values of order parameters ($\pm 10\%$ of the values above), E_{tg} (between 400 and 1000 cal/mol), and the dihedral angle for a *gauche* rotation (between 112° and 120°). The highest and lowest values of each dipolar coupling resulting from these calculations provided the search ranges for the dipolar couplings. These ranges seemed consistent with spectra that reproduce reasonably the overall features and width of the experimental one.

We then started a fit using the GA which was successful in unraveling the spectrum of the nine-spin system *p*-bromo-biphenyl.²⁰ The details of the method can be found in the paper of Meerts and Schmitt.²⁵ Initially this was unsuccessful for the spectrum of pentane in 1132 for a number of reasons. The uncertainties in some of the larger dipolar constants were still quite large. As a consequence the GA procedure was not able to converge. Probably the main reason for this failure comes from the fact that the GA procedure is a global optimizer without any strategy. If the search ranges become too large there is insufficient coverage of the parameter space to locate the global minimum. We solved this problem initially by breaking up the uncertainty regions for a number of parameters into regions so small that we were certain the GA would converge. The size of the smaller search regions was determined with test spectra. This was done for the dipolar couplings with the largest uncertainties.

Now a grid search was set up in which the GA was applied in the different subregions, until a subregion was found for which the GA succeeded in reproducing the experimental spectrum. In this way we obtained a calculated spectrum that was essentially a perfect fit to the experimental one.

It is clear that this grid search process rapidly will become very expensive. If, for example, n uncertainty regions are divided into k subregions the size of the grid will be k^n , a number that increases steeply with n and k . We therefore decided to implement different EAs. From the literature²⁶ it is known that evolutionary strategies (ESs) can be very successful in improving the search for an optimal solution.

The evolution strategy algorithm starts with one or more parents. A parent is a trial solution that corresponds to a vector of parameters like in the GA, with the difference that each vector has an extra set of parameters corresponding to the type of strategy used. This parent generates offspring. The quality or performance of these children is checked and, depending on the strategy, the next parent is generated. There are several different strategies for the generation of the offspring as well as the generation of the next parent. For a more detailed description see Ref. 26.

The offspring is generated from the parents in a mutative step-size fashion. The magnitude and direction of the steps are dependent on previous history and on the quality of offspring generated. A drawback of the standard ES is the fact that the mutations of the decision and the strategy parameters are subject to independent random processes. If, for example, an individual with a large step size undergoes only a very small change in the decision parameters, and this small change turns out to yield a high fitness, the large step size will be inherited by the next generation. As a result, the fitness in the next mutations may worsen. This problem is resolved in derandomized (DR) algorithms which make the random mutations in decision and strategy parameters dependent on each other.

This idea was implemented first as DR1 and soon improved by the concept of accumulated information,²⁷ which is called DR2. The history of the optimization is recorded and the evolution of the mutation ellipsoid is partially governed by past successful mutations.

A further improvement was achieved by Hansen and Ostermeier²⁸ with the covariance matrix adaptation ES (CMA-ES). It turns out to be a particularly reliable and highly competitive EA for local optimization and, surprisingly at first sight, also for global optimization.²⁹ The CMA-ES does not leave the choice of strategy parameters open to the user—only the population size can be set. Finding good strategy parameters is considered to be part of the algorithm design.

Figure 3 depicts the first four generations of an ES and demonstrates the effect of the chosen strategy. In general, the ESs converge faster and are more robust than the GA.

Next we implemented this algorithm into our program, selected the estimated uncertainty regions discussed above for the dipolar couplings, and performed a fit of the spectrum of pentane in magic mixture. This spectrum was even harder to deal with than that of pentane in 1132 because the density of lines is larger (smaller dipolar couplings) and the signal-

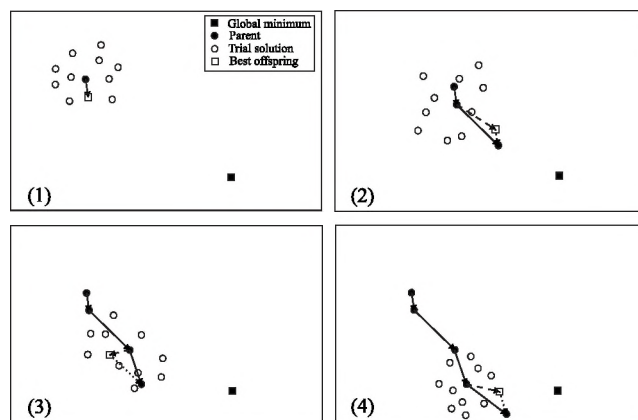


FIG. 3. The first four generations of an ES: (1) an initial population is generated, and the best offspring is used as the next parent. (2) The offspring is spread over a larger area in the second generation due to the relatively large step made in the previous generation. The vector from the parent to the best offspring (dashed line) is combined with the (shortened) mutation vector of the last generation (dotted line) to generate the new parent (solid line). (3) Due to the correlation between the past two mutations the search range has been extended again in the general direction of both mutations while it has been limited in the perpendicular direction. The best offspring is now a local minimum. The memory effect of the EA, which incorporates past mutation vectors into the calculation of the next parent, helps to overcome the local minimum and the next parent is still closer to the global minimum. (4) The barrier between the local and global minima has been overcome, and the optimization is progressing toward the global minimum.

to-noise was smaller, while at the same time the background removal was more difficult. It turned out that the CMA-ES procedure was immediately successful in obtaining an essentially perfect fit of the observed spectrum and determining the parameters unambiguously. At that point there was only one small glitch left. Due to the large density of lines it was relatively difficult to remove completely the background from the observed spectrum. In spite of this the CMA-ES succeeded in fitting all parameters and transitions. Improvement of the accuracy in the final values of the parameters can be realized by a better way of dealing with the background. With our initial fit we were in the position to carry out an almost perfect removal of the background from the experimental spectrum. This was performed in the following way. The calculated spectrum resulting from the CMA-ES was subtracted from the experimental one. To remove the high-frequency noise in this difference a degree of smoothing was applied. Finally this smoothed difference was subtracted from the original experimental spectrum. In this way an experimental spectrum was obtained which is almost background-free. The result for part of the spectrum is shown in Fig. 4. Using this approach we were able to improve the parameters in a final CMA-ES fit. In addition, it was possible to determine accurately the small indirect spin-spin couplings $J_{\mu\nu}$ between nuclei μ and ν .

The background removal procedure as described in the previous paragraph was repeated for the spectrum of pentane in 1132 (see Fig. 2), and again we were able to fit the indirect spin-spin coupling constants. Within estimated error the results for the $J_{\mu\nu}$ for pentane in 1132 and in magic mixture are identical, as expected.

What is left to calculate are the statistical errors in the parameters. This is, in general, a difficult task for EAs. How-

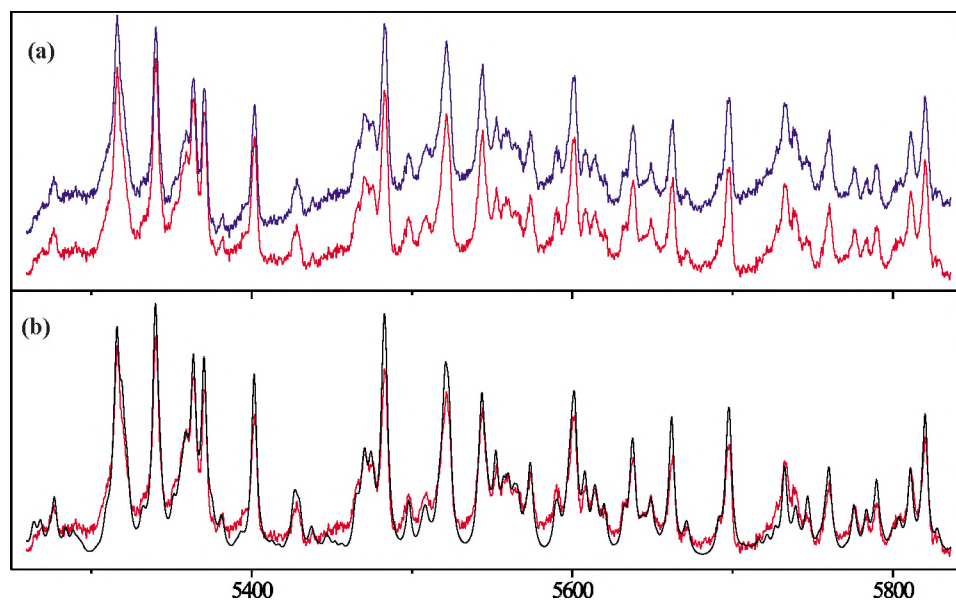


FIG. 4. (Color online) A small part of the ^1H NMR spectrum of pentane dissolved in magic mixture. This shows the effect of the background removal method discussed in the text. (a) The upper and lower traces show the experimental spectra without and with background removal, respectively. (b) The noise-free trace is the calculated spectrum and the other the experimental spectrum after removal of background. All horizontal scales are in Hz.

ever, in our case it is relatively simple. The converged result of the EA not only produces the best values for the fit constants but at the same time the quantum numbers of the individual transitions are assigned. This allows a classical least-squares fit using the assigned frequencies. In Meerts and Schmitt²⁵ this is called an “assigned fit,” and the definitions of the statistical errors and correlation coefficients are discussed in Appendix B of that paper. The errors reported in Table I are based on a 3 Hz uncertainty in the experimental line positions.

In summary, it is apparent that the combination of the relatively crude CI model to estimate conformer order parameters with sophisticated EA fitting methods is the key to solving extremely complicated NMR spectra of solutes in liquid-crystal solvents.

V. RESULTS AND DISCUSSION

The experimental intra- and intermethylene dipolar couplings obtained from the EA fitting are listed in Table I.

There are 11 dipolar couplings available from the analysis of the spectrum of pentane in each liquid crystal. The challenge is to translate these very accurate dipolar couplings into similarly accurate information about the structure, orientational order, and relative probabilities of the various pentane conformers. In this paper we present a preliminary analysis that contains the main features of the problem, but does not exploit fully the high accuracy of the experimental dipolar couplings.

The experimentally determined indirect spin-spin couplings ($J_{\mu\nu}$) are given in the caption of Table I. We note that the assignment of intermethylene couplings is not *a priori* obvious, i.e., nuclei 6 and 7 in Fig. 1 can be interchanged without affecting the experimental spectrum. However, applying the well-known Karplus relationship ($^3J_{\text{HH}}$ (in Hz) = $8.02 - 1.2 \cos \phi + 7.1 \cos 2\phi$) (Ref. 30) to the indirect couplings and averaging over the relevant conformations as discussed below, the assignment of Table I is confirmed.

TABLE I. Dipolar couplings (in Hz) for pentane. The indirect couplings are equal within experimental error in the two experiments. The values (in Hz) determined from the magic mixture and 1132 spectra are, respectively, $J_{14} = 7.39 \pm 0.13$, 7.45 ± 0.09 ; $J_{16} = -0.22 \pm 0.07$, -0.23 ± 0.06 ; $J_{18} = 0.01 \pm 0.12$, 0.00 ± 0.09 ; $J_{45} = -13.2 \pm 0.4$, -13.3 ± 0.3 ; $J_{46} = 5.9 \pm 0.2$, 5.7 ± 0.2 ; $J_{47} = 9.2 \pm 0.2$, 9.4 ± 0.2 ; $J_{48} = -0.4 \pm 0.2$, -0.4 ± 0.2 ; $J_{49} = -0.3 \pm 0.2$, -0.2 ± 0.2 ; and $J_{67} = -13.2 \pm 0.4$, -13.1 ± 0.3 . The values $J_{46} = 6.21$ Hz and $J_{47} = 11.11$ Hz [estimated from the Karplus relationship (Ref. 30) using the GAUSSIAN 03 conformer structures and the conformer probabilities reported in this table] agree with the experimental values.

Solvent	Conformer	D_{45}	D_{46}	D_{47}	D_{48}	D_{49}	D_{67}	Probability
Mixture	<i>tt</i>	2898.8	182.3	245.6	-1784.6	-520.4	2893.4	0.350
	<i>gt</i>	1800.1	-67.9	-92.8	-156.8	-571.9	1770.6	0.520
	<i>g+g-</i>	1098.6	-141.1	-9.6	-331.8	-107.0	483.9	0.038
	<i>g+g+</i>	-460.1	-225.4	54.9	-14.4	27.8	679.8	0.092
	Weighted avg.	1948.6	2.2	42.3	-719.3	-480.8	2013.2	
	Experiment	1944.80(9)	-23.83(13)	50.28(14)	-741.62(10)	-483.06(11)	2037.04(13)	
1132	<i>tt</i>	3777.1	276.2	334.3	-2401.0	-707.2	3770.1	0.346
	<i>gt</i>	2356.8	-92.0	-118.6	-215.8	-780.0	2354.9	0.510
	<i>g+g-</i>	1358.6	-205.5	-29.8	-454.9	-150.1	757.3	0.053
	<i>g+g+</i>	-624.1	-316.7	74.6	-22.4	35.2	942.5	0.091
	Weighted avg.	2524.6	9.1	60.4	-967.2	-647.4	2632.0	
	Experiment	2520.83(7)	-17.95(11)	68.71(11)	-987.68(9)	-650.01(9)	2657.48(11)	

In the simplest RIS approximation²¹ pentane exists in nine different conformers, with four being independent and the additional five related to the four independent ones by symmetry. In general, the order matrices of the four independent conformers are not related to each other.³¹ To describe their orientational order in a uniaxial environment requires 13 independent order parameters, as well as knowledge of the accurate structure of each conformer and its relative probability. Hence the problem looks hopeless, mainly because, as shown by Eq. (3), the observables D_{ij} all involve the product of probability times order parameter. It is not possible to separate these two quantities without making assumptions. In general, we are interested in determining conformer probabilities (which depend on the energy difference between conformers) and how the solvent, including orientational effects, affects them. Thus, in order to make progress it is essential to have some means of obtaining information about conformer order parameters.

There exists no general agreement on the precise nature of U_i^{aniso} . However, models of this potential have been applied to results from NMR experiments. For solutes in magic mixtures the CI model²⁴ is especially successful for a wide range of solutes, which is why we employ this model in conjunction with the EA fitting above. The chord model,^{7,8,32} which was designed especially for saturated hydrocarbons, is particularly useful for alkanes, which is why we shall use it for the interpretation of the dipolar couplings.

The chord model describes alkanes as connected CH_2 building blocks. In its simple form these blocks have axial symmetry and there is one adjustable parameter \tilde{w}_0 ; the assumption of biaxiality leads to a second independent parameter \tilde{w}_1 . Here we use the two-parameter version of the chord model with the definitions of \tilde{w}_0 and \tilde{w}_1 as given in Ref. 7.

The experimental dipolar couplings can now be fitted. We restrict our analysis to rigid RIS conformers and fix symmetry-unrelated conformer geometries to those calculated from GAUSSIAN 03 using B3LYP/6-311++G** (Ref. 33) with geometry optimization. In the fit we adjust the two chord parameters \tilde{w}_0 and \tilde{w}_1 . We also adjust the two internal energy parameters: E_{tg} (the energy difference between a *trans* and *gauche* RIS state) and $E_{\text{extra}}(g_+g_-)$ (the extra energy resulting from the strong steric hindrance between methyl groups in the g_+g_- state—the pentane effect). Finally, we adjust the methyl CCH angle.

Allowing the methyl CCH angle to vary does not seem to be in keeping with the spirit of the rigid approximation of Eq. (3). However, detailed studies of methyl groups in methyl fluoride, methyl iodide, and ethane have shown that vibration-reorientation interaction effects are transferable among methyl groups in different molecules, and when neglected lead to an apparent increase in the methyl HCH angle.^{34–36} Large rms values are obtained unless a similar distortion is included in the pentane analysis. Except for this crude inclusion of vibration-reorientation effects, other small-amplitude vibrational effects are neglected.

The 11 dipolar couplings are now fitted with the five adjustable parameters described above, and rms values of 7.6 Hz for magic mixture and 7.0 Hz for 1132 are obtained. The inter- and intramethylene dipolar couplings recalculated from

TABLE II. Fitted parameters for pentane. Numbers in parentheses indicate uncertainties in least significant digits. The parameters \tilde{w}_0 and \tilde{w}_1 reported here correspond to the original definitions in Ref. 7. Note that the values reported in Ref. 5 should be multiplied by a factor $\sqrt{2/3}$ in order to compare them to the theory of Ref. 7.

Parameter	Magic mixture	1132
\tilde{w}_0 (cal mol ⁻¹)	133 (1)	162 (1)
\tilde{w}_1 (cal mol ⁻¹)	150 (4)	215 (3)
E_{tg} (cal mol ⁻¹)	573 (10)	547 (7)
$E_{\text{extra}}(g_+g_-)$ (cal mol ⁻¹)	547 (169)	350 (83)
CCH angle decrease (deg)	2.6 (0.2)	2.3 (0.1)

these fit parameters are presented for each conformer in Table I along with conformer probabilities and the experimental and the recalculated weighted average of the conformer dipolar couplings. The large variation in magnitude (and even sign) of dipolar couplings with conformer change demonstrates unambiguously the need for a reliable description of the interaction potential, both for the EA fitting and for the conformational analysis.

The values of the five fitting parameters are presented in Table II. The value $E_{tg} \approx 550$ cal/mol agrees with current wisdom for this energy and is consistent with values obtained for butane⁵ and longer chain alkanes^{9,37} from NMR experiments analyzed using the chord model. The extra energy associated with the second *gauche* rotation in the g_+g_- conformer, which is expected to exhibit strong steric hindrance of the methyl groups, is ≈ 400 cal/mol. This is consistent with the well-known pentane effect.

The parameters \tilde{w}_0 and \tilde{w}_1 obtained for the chord model are close to equal. Literature values of these parameters for other alkanes indicate that they increase with chain length. This increase might provide a clue toward modifications to the model that might give a more accurate description of conformer order parameters—such an improved model might provide a means of exploiting the very high accuracy of the experimental dipolar couplings.

In view of the approximations made, the fits are reasonable but not perfect. However, the quality of the present fits indicates that the overall features of the underlying model are correct. The differences should provide an opportunity to achieve a more complete description of this intriguing problem, including conformer order parameters and conformer energy differences.

VI. CONCLUSIONS

In this paper we have demonstrated that a relatively crude model (the CI model for short-range size and shape interactions between solute and liquid-crystal solvent) for estimating solute conformer order parameters, in conjunction with EA analysis of the experimental NMR spectrum, is quite successful in analyzing the ridiculously complicated spectrum of pentane dissolved in two different nematic liquid-crystal solvents. This represents a fundamental breakthrough in spectral analysis of such complicated spectra and

offers the possibility of extending such investigations to more complicated spin systems than ever envisaged in the past.

We have demonstrated that EA analysis provides very accurate dipolar couplings, indirect spin-spin couplings, and chemical shifts directly from the high-resolution NMR spectrum. There is no need for tedious deuteration nor is it necessary to first analyze multiple-quantum spectra (if that were possible) in order to provide starting parameters that are sufficiently close to the correct ones in order to facilitate analysis of the 1-*Q* high-resolution spectrum. In addition, all dipolar couplings are obtained to very high accuracy which opens the possibility of leading to new insights into problems such as those presented by the conformational averaging discussed here.

The use of the EAs opens up the possibility of analyzing spectra that before were far too complicated, and for which analysis would not be possible with the traditional “line-assignment” technique. The NMR spectra of orientationally ordered pentane (with of order 20 000 transitions) provide excellent examples.

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